Kip3p, interacting with microtubule filaments in the ADP state. Using single molecule fluorescence we found that that the diffusion coefficient was 5400 ± 1400 nm^2/s with an average lifetime on the microtubule lattice of 8 s. Using an optical trap to drag a microsphere coated with Kip3p along microtubules, we measured a single molecule drag coefficient of 790 ± 230 nNs/m at low speeds. Thus we verified the Einstein-Smolukowski relation. For larger speeds and drag forces, we measured a non-linear force-velocity relation which was well fit by a model in which Kip3p is diffusing in a periodic potential with an 8-nm periodicity and a barrier height between binding sites of 14 ± 2 *kT*. This finding of an 8-nm periodicity is supported by an analysis of the positional fluctuations. Our measurements are a step towards resolving the molecular mechanism underlying protein friction an important parameter for active protein locomotion limiting the efficiency.

2618-Pos Board B588

Role of cortical rigidity in spindle positioning in C. elegans

Jacques Pecreaux, Stefanie Redemann, Anthony A. Hyman, Jonathon Howard.
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

The first cell division in C. elegans is asymmetric. Asymmetric cell division requires correct positioning of the mitotic spindle. Prior to metaphase, the nuclear-centrosome complex, the precursor of the mitotic spindle, is positioned in the cell center. During anaphase, the spindle is displaced towards the posterior so that bisection of the spindle during cytokinesis leads to daughter cells of unequal sizes. Forces that center and position the spindle come from cortical force generators that pull on astral microtubules. In order to generate force, the cortex needs to provide a stiff anchoring platform. However, a role for the cortex in C. elegans has only been described with respect to polarity establishment. We perturbed the acto-myosin cortex by RNAi of non-muscle-myosin II (nmy-2) using conditions that allowed us to avoid disturbing polarity. Strikingly, in nmy-2(RNAi), membrane tubes are pulled from the plasma membrane into the cell. They were seen after RNAi against other actin cytoskeleton proteins and members of force generation complex, suggesting that the cortical force generators pull the invaginations, and a weakening of the cortex. As expected, we observed an increase in the variance of spindle position and orientation in nmy-2(RNAi). We used the oscillations of the centrosomes during anaphase as a reporter of spindle mechanics, and measured an increase in oscillations frequency but only a marginal decrease in amplitude. In order to understand this phenotype, we used our previously published model to analyze the results. Only by including the cortex into the model, we were able to fully describe the role of NMY-2. In summary, the occurrence of tubes after nmy-2(RNAi) strongly points towards a weakening of the cortex and the analysis of the spindle positioning suggests that the cortex provides a rigid platform for anchoring the force generators.

2619-Pos Board B589

Microtubule Binding and Rotation of the Kinesin-14 Stalk

Sharyn A. Endow, Zhang-Yi Liang, Mark A. Hallen.
Duke Univ Med Ctr, Durham, NC, USA.

Movement of motors along cytoskeletal filaments is thought to be driven by small structural changes that are amplified by a large rotation of the alpha-helical coiled coil. The coiled-coil stalk of the kinesin-14 motor, Ncd, has been visualized in a rotated conformation in a crystal structure and proposed to act like a lever to amplify force produced by the motor, resulting in a working stroke that directs the motor to the microtubule minus end. We show here that an Ncd mutant that is trapped in a stalk-rotated conformation binds tightly to microtubules and shows fluorescence resonance energy transfer between the end of the stalk and microtubule, indicating that rotation of the stalk towards the microtubule is coupled to motor binding to microtubules. A mutant blocked in stalk rotation binds weakly to microtubules and shows no energy transfer, demonstrating that binding by the motor to microtubules requires movement of the stalk. Energy transfer assays show that wild-type Ncd binds to microtubules without added nucleotide with the end of the stalk more than ~9 nm from the microtubule, rotating less than 50 degrees from a position perpendicular to the microtubule. Upon binding with a non-hydrolysable ATP analogue, the stalk lies within ~6 nm of the microtubule surface, representing a rotation of ~70 degrees. These findings are consistent with previous reports by cryoEM that the Ncd stalk rotates when the microtubule-bound motor binds ATP. However, our results indicate that stalk rotation is initiated by filament binding and ADP release, and completed upon ATP binding, rather than triggered by ATP binding. Initiation of the Ncd stalk rotation by microtubule binding and ADP release, and its completion on ATP binding is reminiscent of the two-step working stroke of myosin I, revealing an unexpected similarity between the motors.

2620-Pos Board B590

A Common Microtubule Activation Mechanism for Plus- and Minus-End Directed Kinesin Motor Proteins

Charles V. Sindelar[1], Kenneth H. Downing[2].
[1]Brandeis University, Waltham, MA, USA, [2]Lawrence Berkeley National Laboratory, Berkeley, CA, USA.

Interactions with the microtubule drive the working cycle of all kinesins, yet the the structure of the kinesin-microtubule complex remains poorly characterized. We solved a series of 8Å-resolution cryo-EM structures representing the microtubule-bound hydrolysis cycles of two different kinesin family members: plus-end directed conventional kinesin, and minus-end directed NCD. These structures reveal that microtubule binding transforms part of the poorly ordered loop L11, located within the switch II nucleotide response element of these kinesins, into a stable extension of the so-called "switch II helix." We show how this extension likely enables the helix to function as a rigid "relay" element, driving the "power stroke" of both conventional kinesin and NCD. Moreover, binding of ATP analogs in either kinesin variant was associated with a 3% lengthwise contraction of the microtubule lattice. This latter effect may link conventional kinesin and NCD to the microtubule-depolymerizing kinesins.

2621-Pos Board B591

Mechanistic Analysis of Kar3Cik1 for Mitotic Function

chun ju chen, **Susan P. Gilbert**.
RPI, Troy, NY, USA.

Kar3Cik1 is a *S. cerevisae* Kinesin-14 motor protein that promotes microtubule (MT) shortening during karyogamy yet acts to crosslink interpolar MTs (ipMTs) during anaphase. The Kar3 head contains both an ATP and MT binding site, yet there is no nucleotide binding site in Cik1. Presteady-state and steady state experiments have been pursued to define the mechanism by which Kar3Cik1 performs its mitotic function to crosslink and stabilize antiparallel ipMTs. We have developed an approach to begin our experiments with a homogenous population in which the Cik1 head binds to the MT first followed by the Kar3 motor domain (Kar3MD). The MT association kinetics at 2.6 $\mu M^{-1}s^{-1}$ are fast followed by Kar3MD association and rapid ADP release at 26 s^{-1}. ATP binding to the Kar3MD is also a fast step at 4 $\mu M^{-1}s^{-1}$ with $k_{off} = 12$ s^{-1}. Dissociation of the MT-Kar3Cik1 complex occurs as a slow step at 3.8 s^{-1}. These initial results suggest a model in which Kar3-Cik1 interacts with the MT through an alternating cycle of Cik1 binding followed by Kar3MD binding. Because Cik1 does not have a nucleotide binding site, we propose that head-head communication is mediated by a strain-dependent mechanism.

Supported by NIH grant GM54141 and NIH Career Development Award K02-AR47841 to SPG.

2622-Pos Board B592

Multi-functional Dynamic Control of Bipolarity, Chromosome segregation and Spindle Elongation by a Novel Essential Chromatin Binding Klp in Fission Yeast

Erika Sadeghi[1], Jason Patregnani[2], Adrianna S. Rodriguez[3], **Janet L. Paluh**[1].
[1]Rensselaer Polytechnic Institute, Troy, NY, USA, [2]Connecticut Childrens Medical Center, Hartford, CT, USA, [3]NYU School of Medicine, New York, NY, USA.

A subset of all known kinesin-like microtubule motor proteins (Klps) regulates spindle function for fidelity in chromosome segregation. While some families are ubiquitous, such as Kinesin-5 and Kinesin-14 Klps, others families seem restricted to multi-cellular eukaryotes, such as chromokinesins and MKlp1-like passenger proteins. We report a novel essential chromatin-binding Klp in fission yeast *Schizosaccharomyces pombe* Ckk1 (Cho1 and Kid-like kinesin). *S. pombe* Ckk1p has an N-terminal motor domain and carboxy-terminal tandem basic-Zip DNA binding domains. It localizes to chromosome arms in prometaphase and kinetochores in metaphase. In prometaphase to anaphase it also associates with overlapping anti-parallel microtubules of the spindle midzone. Passenger proteins show dynamic relocalization from chromosome arms to kinetochores to spindle midzone and regulate cytokinesis. Although Ckk1p has the conserved Cho-domain, it does not have actin binding domains and exhibits no cytokinesis defects.